

Supplementary material and methods

Ti-IMAC beads synthesis and quality control steps

The Ti-IMAC microsphere synthesis was conducted according to the protocol by Zhou *et al*, who produced a highly hydrophilic enrichment matrix, which was proven to reduce unspecific binding, and to be chemically stable at extreme pH. The protocol comprised five synthetic steps including bead polymerization, covalent binding of the linker and the chelating ligand, and loading with Ti^{4+} -ions. A description of the protocol is given below.

1. Synthesis of monodisperse polystyrene seed microspheres. PVP (1.25 g) and Triton X-100 (1.14 ml) were dissolved in ethanol (64 ml). A solution of AIBN (0.58 g) in styrene monomer (16 ml) was added. The mixture was stirred 200 rpm and 70°C for 24 h. The reaction mixture was then centrifuged at 3000 rcf for 5 min and the supernatant was discarded. The obtained monodisperse polystyrene microspheres were washed with ethanol (3 x 20 ml) and then dried under vacuum. Monodispersity and size distribution of the polystyrene microspheres were verified under the light microscope.

2. Synthesis of poly(GMA-co-TMPTMA) monodisperse microspheres. A solution of PVA (2 g) and SDS (0.5 g) in ELGA water (200 ml) was prepared. To 15 ml of this solution 0.45 g of the polystyrene seed microspheres obtained in step 1 of the synthesis protocol were added. The suspension was exposed to sonication. To a solution of the monomers GMA and TMPTMA (each 6.7 ml) in toluene (16.6 ml) was added 0.15 g of AIBN. 150 ml of the aqueous PVA/SDS solution was added. The mixture was emulsified by sonication and then added dropwise to the microsphere suspension under mechanical stirring (200 rpm). The mixture was kept at 30°C for 20 h and then raised to 70°C for 24 h. The mixture was centrifuged at 300 rcf for 5 min and the supernatant was discarded. The poly(GMA-co-TMPTMA) microspheres were washed with THF (3 x 20 ml) and acetone (3 x 20 ml) and then dried under vacuum. Diameter and monodispersity was again verified under the light microscope.

The irregular by-product which was produced during this reaction was removed by resuspending in water (30 ml) and sedimentation of the microspheres for 5 min. The supernatant was discarded and the process was repeated twice. Microspheres were then centrifuged for 5 min at 3000 rcf and the supernatant was discarded. The pellet was dried under vacuum.

3. Preparation of poly(GMA-co-TMPTMA-NH₂) microspheres. An excess of anhydrous ethylenediamine (150 ml) was added to 7 g of poly(GMA-co-TMPTMA) microspheres. The mixture was stirred at 200 rpm and 80°C for 3 hours under an atmosphere of argon. The microspheres were centrifuged (3000 rcf, 5 min) and the supernatant was discarded. The pellets were washed with water (3 x 20 ml) and ethanol (3 x 20 ml). Successful binding was verified by ninhydrin assay:

Ninhydrin Assay

To suspensions of 1 mg poly(GMA-co-TMPTM), poly(GMA-co-TMPTMA-NH₂) and poly(GMA-co-TMPTMA-PO₃H₂) microspheres in 0.5 ml n-butanol was added 0.5 ml 3% ninhydrin in n-butanol. The mixtures were heated to 90°C on a thermo mixer with shaking for 30 min. The amount of blue-colored Ruhemann's Purple produced was measured in a photometer at 570 nm. The results were compared with a dilution series of known concentrations of ethylamine in n-butanol. Following concentrations were prepared: 0.1 mM, 0.2 mM, 0.4 mM, 0.8 mM, 1.6 mM, 3.2 mM, 6.4 mM, and 12.8 mM. To 0.5 ml of each concentration was added 0.5 ml of 3% ninhydrin in n-butanol. The mixtures were heated and measured like the microsphere samples (see above).

4. Preparation of poly(GMA-co-TMPTMA-PO₃H₂) microspheres. 7 g of the poly(GMA-co-TMPTMA-NH₂) microspheres were dispersed in 100 ml of water. To the dispersion, phosphorous acid (5.1 ml), fuming hydrogen chloride (10 ml) and formaldehyde (8 ml) were added. The mixture was stirred at 150 rpm at 100°C for 24 h and then centrifuged (3000 rcf for 5 min). The microspheres were washed with ethanol (3 x 20 ml) and water (3 x 20 ml) and then dried under vacuum. The reaction product is displayed in. Successful reaction was tested by molybdenum blue assay:

Molybdenum Blue Assay

To 1 mg of poly(GMA-co-TMPTMA-NH₂), poly(GMA-co-TMPTMA-PO₃H₂) and poly(GMA-co-TMPTMA-PO₃H₂-Ti⁴⁺) microspheres in 1.5 ml reaction tubes was added 1 ml of molybdenum(VI) oxide solution in H₂SO₄. The mixtures were vortexed and incubated at room temperature for 5 min. The tubes were spun down in the table top centrifuge for 10 s and the color of the microsphere pellet was examined.

5. Immobilization of Ti⁴⁺ on the phosphonate group of the monodisperse microspheres. 100 mg of poly(GMA-co-TMPTMA-PO₃H₂) were incubated with Ti(IV) chloride solution (20 ml) for 8 h under mechanical stirring at 150 rpm. The resulting mixture was centrifuged and the supernatant was discarded. The microspheres were washed with 30% ACN/ 0.1% TFA (3 x 20 ml) and stored in 50% ethanol.